

Figure 10 is a schematic illustrating object planes associated with the imaging systems, as shown in Figures 6-9.

Figures 11 and 12 are schematics illustrating an alternative implementation of a component of the imaging system, as shown in Figures 2-3 and 6-9.

5 Figures 13-14 are schematics illustrating an alternative implementation of the imaging system, as shown in Figures 2-3 and 6-9.

Figure 15 is a schematic illustrating an alternative implementation of the imaging system, as shown in Figures 2-3, 6-9, and 13-14.

Figure 16 is a schematic illustrating an exemplary set of images on a
10 detector of the imaging system, as shown in Figures 2-3, 6-9, and 13-14.

Figures 17-18 are schematics illustrating an alternative implementation of the imaging system, as shown in Figures 2-3, 6-9, 13-15.

Figure 19 is a schematic illustrating an exemplary set of images on a detector of the imaging system, as shown in Figures 17-18.

15 Figures 20-21 are schematics illustrating an alternative implementation of the imaging system, as shown in Figures 2-3, 6-9, 13-15, 17-18, and 20-21.

Figure 22 is a schematic illustrating object planes associated with the imaging system, as shown in Figures 20-21.

Figure 23 is a schematic illustrating a two-dimensional imaging system
20 using active focusing.

Figure 24 is a schematic illustrating an exemplary set of images projected on one of the detectors of the two-dimensional imaging system, as shown in Figure 23.

Figure 25 is a schematic illustrating an alternative implementation of the imaging system, as shown in Figures 2-3, 6-9, 13-15, 17-18, and 20-21.

25 Figure 26 is a flowchart illustrating a method used by alternative implementation of the imaging system, as shown in Figure 25.

Figure 27 is a schematic illustrating an alternative implementation of the imaging system, as shown in Figures 2-3, 6-9, 13-15, 17-18, 20-21 and 25.

Figure 28 is a schematic illustrating an alternative implementation of the imaging system, as shown in Figures 2-3, 6-9, 13-15, 17-18, 20-21, 25, and 28.

DETAILED DESCRIPTION OF THE INVENTION

Described herein are systems and methods for achieving and maintaining
5 focus of target objects subject to low-light, high-resolution imaging. In general, light either reflecting, scattering or emanating from a target object is collected and split into two or more light components. The optical power levels of some, but not all the light components are then modified such that when the light components are recombined with an angular separation to form an image, each of the light components have differently positioned
10 image planes where an object point of the target object is imaged.

For each image plane pair, a detector is such that each detector receives focused images from two object planes at the target object associated with the image plane pair of the detector to increase depth of field and focusing capability. In some implementations, focus is actively maintained through computer automated positioning of
15 components. Other implementations actively maintain focus with a feedback arrangement integral to a two-dimensional imaging system.

In the following description, numerous specific details are provided to understand embodiments of the invention. One skilled in the relevant art, however, will recognize that the invention can be practiced without one or more of these specific details, or with other equivalent elements and components, etc. In other instances, well-known
20 components and elements are not shown, or not described in detail, to avoid obscuring aspects of the invention or for brevity. In other instances, the invention may still be practiced if steps of the various methods described could be combined, added to, removed, or rearranged.

25 A method 50 used by implementations of low-light, high-resolution imaging systems is illustrated in Figure 1. The method 50 first collects light from a target object, such as emanating, scattered, reflected, and/or refracted light (step 58). The collected light is then collimated by focusing to infinity (step 62). The collimated light is then split into a

collective total consisting of two or more optical paths (steps 66). Optical power is then added or subtracted from the light in chosen one or more, but not all, of the optical paths of the collective total to defocus the light in the chosen one or more optical paths (step 70). Light in the collective total of optical paths is then recombined wherein light of the one or more chosen optical paths has one or more small angular separations with respect to light of other optical paths of the collective total (step 74).

The recombined light is then focused on one or more detectors resulting in one or more spatial separations of the imaged target object based upon two or more image planes at the imaged target object associated with two or more object planes at the target object (step 78). Images of the imaged target object associated with the two or more image planes are then collected by the one or more detectors for analysis (step 82) and the method ends to be ready for further imaging of other target objects. As an example, if two optical paths make up the collective total, then optical power of only one of the paths is altered so that there is a spatial separation between two images resulting from the two light paths on a detector. For an image plane defined by the detector, there are two conjugate object planes separated along the optical axis of the imaging system. Optical power of the one path is altered to control the axial separation between the object planes so that the depth of field provided by the first image just overlaps the depth of field provided by the second image to extend the total depth of field of the imaging system.

An implementation of an imaging system 100 as shown in Figure 2 is configured to produce multi-focal plane images of a target object 102, such as biological cells or other small particles, being transported by a fluid flow, in the direction of the z-axis of Figure 2, through a flow cell cuvette 104. The imaging system 100 has an unaltered optical path 106 with a collection lens 108, an amplitude beam splitter 110 with a beam splitter optical coating 112, an amplitude beam splitter 114 with a beam splitter optical coating 116, an imaging lens 118, and a first detector 120 (shown in Figure 3). The imaging system 100 also has a defocus optical path 122 with a first reflector 124, a defocus system 126 being a negative lens 128 in the implementation shown, and a second reflector 130 and sharing the collection lens 108, the beam splitter optical coating 112, the amplitude